# Phycoerythrin Signatures in the Littoral Zone

A. Michelle Wood
Dept. of Biology
University of Oregon
Eugene, Oregon 97403
Phone: (541) 346-0454 FAX: (542) 346-2364 email: miche@darkwing.uoregon.edu

Award Number: N00014-99-1-0177
http://www.nrlssc.navy.mil/nrlssclinks.html (click on remote sensing, Code 7340)
http://www.photon.coas.oregonstate.edu/ocean/projects/hycode/hycode_pictures.html

## LONG-TERM GOALS

I am interested in understanding how, and in what ways, the taxonomic composition of the phytoplankton affects ecosystem-level processes in the sea. I am particularly interested in the relationship between the optical environment and the distribution of phytoplankton with different light harvesting pigments. To this end, I have been working in collaboration with experts in remote sensing and ocean optics, toward the development of an "optical biogeography" for marine picoplankton, particularly different spectral forms of marine *Synechococcus* and *Prochlorococcus*. These are globally important organisms for which the long-term goal would be the development of optical signals that could be used to estimate their importance in a given region of the ocean using remote sensing.

## OBJECTIVES

Phycoerythrin (PE) is the principal light-harvesting pigment of marine *Synechococcus*, *Trichodesmium*, the recently described chroococcoid nitrogen-fixing unicellular cyanobacteria common in the open ocean (Zehr, 2001), and cryptomonads. It is also a minor component of the photosynthetic apparatus of some strains of *Prochlorococcus*. Different spectral forms of phycoerythrin harvest light in different regions of the spectrum depending on the relative concentration of two different chromophores that can be incorporated into the assembled PE molecule in different combinations. These chromophores are phycoerythrobilin (PEB, $\lambda_{AbsMAx}$ ~550 nm), found in all PEs, and phycourobilin (PUB, $\lambda_{AbsMax}$ ~500 nm) found in varying concentration in some forms of PE. PEB provides for efficient utilization of green wavelengths of light and PUB enhances absorption of blue and blue-green wavelengths of light which penetrate clear ocean water more efficiently that green and red wavelengths. The cyanobacteria which utilize PE as the principal light harvesting pigments are under very strong selection to synthesize a pigment with a chromophore composition that can efficiently utilize the available wavelengths (Wood, 1985).

PE is a highly fluorescent molecule and PE containing organisms are so abundant in most ocean waters that it is relatively simple to characterize the dominant spectral form of PE in bulk seawater using fluorescence excitation spectroscopy (Wood et al., 1998; 1999). Recent work in the Arabian Sea and coast of North America led me to propose that the PEB-lacking spectral form of PE would predominate in Case II waters, the low PUB spectral form of PE would predominate in "green" [$K_d(440)>K_d(550)$] Case I Waters, and the high PUB spectral form of PE would predominate in "blue" [$K_d(440)<K_d(550)$] Case II Waters. In the Arabian Sea, where we found extremely high growth rates

# Report Documentation Page

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| **30 SEP 2001** | | **00-00-2001 to 00-00-2001** |

| 4. TITLE AND SUBTITLE | 5a. CONTRACT NUMBER |
|---|---|
| **Phycoerythrin Signatures in the Littoral Zone** | 5b. GRANT NUMBER |
| | 5c. PROGRAM ELEMENT NUMBER |
| 6. AUTHOR(S) | 5d. PROJECT NUMBER |
| | 5e. TASK NUMBER |
| | 5f. WORK UNIT NUMBER |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Dept. of Biology,University of Oregon,,Eugene,,OR, 97403** | 8. PERFORMING ORGANIZATION REPORT NUMBER |
| 9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) | 10. SPONSOR/MONITOR'S ACRONYM(S) |
| | 11. SPONSOR/MONITOR'S REPORT NUMBER(S) |

12. DISTRIBUTION/AVAILABILITY STATEMENT
**Approved for public release; distribution unlimited**

13. SUPPLEMENTARY NOTES

14. ABSTRACT

**am interested in understanding how, and in what ways, the taxonomic composition of the phytoplankton affects ecosystem-level processes in the sea. I am particularly interested in the relationship between the optical environment and the distribution of phytoplankton with different light harvesting pigments. To this end, I have been working in collaboration with experts in remote sensing and ocean optics, toward the development of an ???optical biogeography??? for marine picoplankton, particularly different spectral forms of marine Synechococcus and Prochlorococcus. These are globally important organisms for which the long-term goal would be the development of optical signals that could be used to estimate their importance in a given region of the ocean using remote sensing.**

15. SUBJECT TERMS

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a REPORT | b ABSTRACT | c THIS PAGE | | | |
| **unclassified** | **unclassified** | **unclassified** | **Same as Report (SAR)** | **6** | |

for phycoerythrin-containing picocyanobacteria (Publication 1, See below), we also found that the low PUB-containing form of PE predominated in upwelling influenced waters and replaced the high PUB-containing form of PE in offshore waters as the SW Monsoon progressed (Wood et al., 1999 and unpublished).

The objectives of this project are: 1) to determine whether or not the proposed "optical biogeography" for organisms synthesizing different spectral forms of PE is robust across a wide range of optical environments in different parts of the world ocean, 2) to determine if there is a correlation between the spectral signature of PE in different environments and the inherent and apparent optical properties of those environments, 3) to determine if there are optical signals that can be used to predict the relative importance of PE-containing organisms in the phytoplankton, and 4) to understand the biological mechanisms that underlie changes in the spectral signature of PE in a water mass.

## APPROACH

In order to address objectives 1-3, I am primarily working in the field. This approach involves measurement of the PE spectral signature of bulk water using fluorescence spectroscopy and determining the correlation of PE spectral type and the concentration of PE-containing organisms with a range of optical parameters. The research is being coordinated with collaborators who are funded independently to make optical measurements: S. Pegau, R. Zaneveld, T. Cowles, R. Arnone, R.Gould, Alan Weideman, C. Davis, S. Lohrenz, D. Johnson, C.Trees, J. Mueller, H. Maske. I am very grateful for their willingness to contribute to this research.

Objective 4 is being addressed with a laboratory component. Previous work with one strain of PUB-lacking *Synechococcus* and three strains that contain different spectral forms of PE has shown that the PUB-lacking form of PE has only one PE, but the strains that have a PUB+ spectral phenotype have two PEs (Glazer, 1999; Ong & Glazer, 1987, Wilbanks et al., 1991). Thus, one hypothesis that underlies this work is that strains with the PUB-lacking PE phenotype will be unable to change spectral signature and are restricted to "green" environments (as predicted by the hypothesis that these forms are diagnostic of Case II waters) and that at least some PUB-containing strains will be able to change spectral signature in response to the changing spectral composition of available light. I maintain a collection of more than 60 strains of PE-containing picocyanobacteria, and additional strains are being obtained from culture collections. These are being grown under conditions of comparable PFD but under different wavelengths of available light to screen for the capacity to change PE spectral signature in response to changes in the spectral composition of available light. I am approaching the question of the presence of multiple copies of PE in different strains, and testing the hypothesis that strains with a PUB-lacking PE phenotype have only one form of PE by using molecular approaches to look at he organization of the PE genes in multiple strains of each spectral type.

## WORK COMPLETED

We have participated in four cruises, two in the Gulf of Mexico, one in the Gulf of California, and one at the LEO-15 site. This has given us data from a wide range of optical environments including some extremely turbid Case II waters off the Mississippi River Plume and on the continental shelf off New Jersey. Fluorescence characterization of the PE signatures from the cruises is complete, and data synthesis is completed for the Gulf of California cruise; data processing for the optical data from that cruise and the first Gulf of Mexico cruise (West Florida Shelf) has also been completed by the cooperating groups (NRL/SSC and Ocean Optics at OSU). We have presented the preliminary findings from these cruises at national meetings this year (Publications 4,5). About 35 strains of

*Synechococcus* have been screened for the capacity to chromatically adapt to changing light regimes and we have developed the capacity for DNA extraction from *Synechococcus* and PCR amplification of the DNA using primers to a region of the 16S rDNA gene (Publications 2,3). With assistance from the laboratory of W. Hess (c.f. Hess et al.1996,1999) and the Univ. of Oregon genomics group (J. Postlethwaite, P.I.), we are designing degenerate primers for PCR amplification of PE genes with varying specificity (to PEI or PEII and to different subunits).

**RESULTS**

Our fieldwork has produced a number of extremely interesting results, specifically:

1) Low PUB-PEs are associated with upwelling influenced water masses.

2) The spectral signature of PUB-lacking PEs appears to be restricted to Case II waters.

3) The spectral signature of high-PUB PEs appears to be a good indicator of introduction of oceanic water onto continental shelf regions.

4) *Prochlorococcus* appears to be an open ocean specialist but PE-containing *Synechococcus* can reach extremely high abundance ($10^5$-$10^6$ $ml^{-1}$) in coastal waters and upwelling-influenced waters. Thus, while *Synechococcus* may be a greater proportion of the total number of phytoplankton cells in the open ocean, they must also be viewed as an important component of the coastal ocean. This is particularly true in tropical and sub-tropical waters where they reach their highest abundance most frequently.

A further, and very exciting, result has come from the Gulf of California cruises where we identified three distinctly different optical environments based on hyperspectral reflectance and the slope of the beam attenuation spectrum. Upon further examination of the data, it was clear that each environment corresponded to water masses dominated by different phytoplankton communities. The three phytoplankton communities were: 1) those typical of open ocean environments where the dominant cells were both *Synechococcus* and *Prochlorococcus*, 2) upwelling-influenced regions with very high *Synechococcus* concentrations, no *Prochlorococcus* detectable by either HPLC or flow cytometry, and very low concentrations of fucoxanthin and other pigments associated with diatoms and dinoflagellates, and 3) more eutrophic stations where large phytoplankton containing chlorophyll c were very abundant.

Our culture studies have revealed the existence of PE-containing *Synechococcus* in the plankton which are completely unrelated phylogenetically to the relatively widely studied strains believed to be representative of the marine picoplankton. One of these, which has a PUB-lacking PE spectral phenotype, is also novel in its physiology with respect to nitrogen storage and can make the nitrogen storage compound, cyanophycin (Publication 3, below). Previous studies had suggested that marine *Synechococcus* lacked this ability. While we have not found any strains of PE-containing picocyanobacteria in our collection that are capable of chromatic adaptation, it is now clear that there is diversity in this character among different strains and that many strains with a PUB+ phenotype cannot chromatically adapt. Our work has been coordinated with similar screening by Brian Palenik's group at Scripps Institute of Oceanography and, to date, of the more than 25 strains screened, only one has shown an ability to chromatically adapt (Wood, unpublished; Palenik, 2001).

## RELATED PROJECTS

Funding for collection of data from the Gulf of California and Oregon Coast has been through NASA SIMBIOS projects to Ron Zaneveld and Scott Pegau, and separately to Jim Mueller and Chuck Trees (CHORS/SDSU). Ship time for the Sea of Cortez was funded through Conayct (Helmut Maske). At the LEO-15 site ship-time and collection of optical data was funded through HYCODE; I anticipate coordination of data analysis and publication of manuscripts with the OSU group (Pegau), NRL/SSC Ocean Color group (Arnone, Gould), and with Curt Davis' group (NRL), as well as other HYCODE participants.

## IMPACT

This work is making it clear that marine *Synechococcus* and other PE-containing organisms are as abundant, or even more abundant, in coastal waters as in oceanic waters. This requires a shift from the paradigm that the microbial loop is mostly important in oligotrophic regions and relatively unimportant in more eutrophic areas. The changing notion of the breadth of the niche for marine *Synechococcus* will require more emphasis on its productivity and fate in nearshore regions as well. Additionally, in some nearshore upwelling regions, they are very abundant and clearly affect ocean color. The generally accepted notion that *Synechococcus* with a high PUB-spectral signature dominate the open ocean appears to be incorrect insofar as it is organisms synthesizing a low PUB-spectral from of PE consistently become the dominant spectral form in Case I environments when there is nutrient enrichment from upwelling or deep mixing. Finally, our data also indicate that the PUB-lacking spectral phenotype of PE is dependably associated with "green" Case II waters. Since it is possible to distinguish PUB+ from PUB- PE spectral signatures using aircraft-borne LIDAR, it might be relatively easy to use this signal to distinguish between "green" Case I and "green" Case II waters over wide geographic areas. This could be extremely useful in determining the suitability of different algorithms for estimating the chlorophyll concentration from remotely sensed data on ocean color.

## REFERENCES


Glazer, A.N. 1999. IN, L. Charpy and A. W. D. Larkum. *Marine Cyanobacteria*. Bul. Inst. Oceanographique. Sp. Number 19. Pp. 419-434.

Hess, W. R., F. Partensky, G. W.M. Van der Staay, J. M. Garcia-Fernanez, T. Borner, and D. Vaulot. *Proc. Nat. Acad. Sci*. 93:11126-11130.

Hess, W. R., C. Steglich, C. Lichtel, and F. Partensky. 1999. Pl. Mol. Biol. 40:507-21.

Ong, L.J. and A. N. Glazer. 1987. *J. Biol. Chem*. 262:6323-6327.

Palenik, B. 2001. *Appl. Env. Micro*. 67:991-94.

Wilbanks, S.M., R. de Lorimer, and A. N. Glazer. 1991. *J. Biol. Chem*. 266:9535-9539.

Wood, A.M., D.A. Phinney, and C.S. Yentsch. 1998. *Mar. Ecol. Prog. Ser*.. 162: 25-31.

Wood, A.M., P.K. Horan, K. Muirhead, D.A. Phinney, C.M. Yentsch, and J.B. Waterbury. 1985. *Limnol. Oceanogr*. *30*:1303-15.

Wood, A. M. 1985. *Nature*. 316:253-55.

Wood, A.M. M. Lipsen and P. Coble, 1999. *Deep-Sea Research II*, 46: 1769-1790.

Zehr, J. 2001. *Nature*. 412:635-638.

**PUBLICATIONS**

1. Sherry, N.S. and A, M. Wood. 2001. Picocyanobacteria in the Arabian Sea during the end of the 1995 Winter Monsoon : diurnal patterns, spatial variability, and division rates. *Deep-Sea Res*., 48:1263-1284.

2. Wood, A. M., S. Miller, W.K.W. Li, and R. Castenholz. 2001. Preliminary study of cyanobacteria, picoplankton, and virioplankton in the Salton Sea with special attention to phylogenetic diversity among eight strains of filamentous cyanobacteria. In Press. *Hydrobiologia.*

3. Wingard, L., S. Miller, J. Selker, E. Stenn, M. M. Allen, and A.M. Wood. Cyanophycin production by a phycoerythrin-containing marine *Synechococcus* from the Arabian Sea. Submitted to *Appl. Environ. Micro.*

4. Wood, A.M., S. Pegau, H. Maske, and J. Mueller. 2001. Association of low-phycourobilin-containing phycoerythrin with upwelling-influenced waters in the Gulf of California. (abstract). *Oceanography* 14:60.

5. Wood, A. M., W. K.W.. Li, R. Arnone, R. Gould, and S. Lohrenz. 2001. Optical biogeography of *Prochlorococcus* and Phycoerythrin-containing picocyanobacteria on the West Florida shelf (abstract). *J. Phycol* 37(suppl):54.

6. Wood, A.M., R. Castenholz, and J. B. Waterbury. 2001. Cyanobacterial systematics in the genomic era (absract). *J. Phycol* 37(suppl):54.